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THE FOCUS ON DRUG DISCOVERY: HOW?

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ABSTRACT: *Science and drug discovery methods is as old as man, so is pathogen transmission and disease spread. It takes little or no effort for diseases to break out but yet it takes the average Scientist decades to find lasting solutions to combat disease.Pathogens need human beings (apart from other vectors like animals) as vehicles to transport them from place to place. Public Enlightenment campaigns can help human beings to minimize disease spread, in addition, reinventing available mechanical constructs, redirecting Scientific experiment towards knowing what essential nutrient or compounds pathogenic organisms need to survive and get established to cause infection in a host in addition to using avirulent bacteria strains as drug delivery tools may probably go a long way in improving bacterial or pathogenic medicine development.*

KEYWORDS: Essential nutrients, avirulent bacteria strains, pathogen transmission , drug discovery focus

INTRODUCTION

"An engineered phage could, in theory, be patented. At the ASM meeting last month, researchers led by synthetic biologist Timothy Lu at the Massachusetts Institute of Technology in Cambridge presented work on a phage engineered to use a DNA-editing system called CRISPR to kill only antibiotic-resistant bacteria. The phage injects the bacterium with DNA, which the microbe transcribes into RNA. If part of the bacterium‘s antibiotic-resistance gene matches that RNA sequence, an enzyme called Cas9 cuts up the cell‘s DNA, killing it.

She adds that doctors in some EU countries send patients‘samples to the Eliava Institute, which then sends back a phage cocktail specific to the bacterium causing the infection. When there‘s no hope, you‘ll do anything, Schmidt says. To lower the chance that resistance will develop, the patients will receive a cocktail of more than a dozen phages that enter bacterial cells in different ways. If the phage treatment fails, patients will then receive standard antibiotics……. Culled from Nature, 2014When there is no hope, "you will do anything", but not anything dangerous I suppose !, Why shouldn't Scientists this century seek alternative approaches which sound or seem less desperate?In my very recent article titled " Pathogen traffickers: Disease causing organisms do not have legs (First issue published online in August, 2014 and recently again in April, 2015), I tried to explain how new approaches to Science may probably help us Scientists all over the world on drug discovery methods which included the issues concerning the reinvention of the Vitek Machine based on my experimental observations and also the use of "Avirulent Bacteria" strains as vehicles to deliver treatment into a sick host", I would like to say again that these bacteria strains are nonpathogenic and considered harmless. I recently ran into two short articles somewhat based on the ideas I was trying to pass across in my last publication (Osayande, JO, 2015). One of

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them is titled "When Bacteria Attack" from the weekly Chemistry alert (ChemistryViews.org - Weekly Alert 9 April 2015), and the other is titled "Nasal Inoculation of the Commensal *Neisseria lactamica* Inhibits Carriage of *Neisseria meningitidis* by Young Adults: A Controlled Human Infection Study" . Please kindly read below.

"**Pathogen traffickers: Disease causing pathogens do not have legs**"

"When disease-causing organisms successfully find themselves into the body of a host, they need a lot of materials (including essential nutrients) to enable them feel comfortable and get established within the host environment. In addition, even in areas of antibiotic resistance, tougher avirulent bacteria strains (which I may like to call "beneficial bacteria") better equipped for survival can be engineered against disease causing bacteria, the only thing we as scientist should concentrate on now is to see;

First and foremost, what compounds or nutrients are essential for the survival of a pathogenic organism or what compounds they need to establish themselves in a host such that in the absence of these essential nutrients (for example) the pathogenic organism is unable to thrive. For instance, iron serves as a signal for biofilm development in *P. aeruginosa* (Banin *et al.*, 2005), and the biofilm serves as a protective covering (some kind of protective shield) against administered antibiotics, so if avirulent strains of the same *P. aeruginosa* or other bacteria species which are better equipped to compete for iron could be engineered and subsequently introduced as a form of treatment into a sick host, the biofilm would not be developed and administered antibiotics will find their way directly to the targeted pathogenic bacteria. I think a great difference will be made, also in the situations of viral infections. It will only boil down to the survival of the fittest organism within a host and the pathogen which is unable to survive, dies"……. (Osayande, JO, 2015)

"When Bacteria attack"

"Autoinducing peptides (AIPs) are cyclic peptides produced by many strains of Staphylococcus bacteria that signal the local density of the bacterial population. Monitoring of the surrounding population (known as quorum sensing) is an important factor for bacteria to "know" when the conditions are right to attack a host organism. AIPs are macrocyclic peptides that have long been known to initiate the production of bacterial virulence factors, i.e., the proteins responsible for harming the host. A team led by Tom W. Muir, Princeton University, NJ, USA, has probed how the size of the macro cycle affects the effectiveness of the AIP. They have synthesized a series of cyclic peptides containing differing amino acids to complete the structure-activity relationship between AIP and its receptor AgrC.

The activity of AIPs with both expanded and contracted macrocycles was assessed by using both in vitro activity assays and cell-based assays. In general, it was found that the macrocycle is highly intolerant to changes in size, but one position was found to be slightly flexible. All of the data from this SAR, and previous studies, was then compiled into a model for receptor activation by AIP. In particular, three points of contact were identified with the AgrC receptor that are critical for triggering downstream effects.

Now that the important residues have been identified, more effective inhibitors of this pathway can be designed and tested. By disrupting the communication between bacterial cells, a new

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type of antibiotic agent could prove valuable in the fight against some of the most virulent strains"…….. authored by Meghan Campbell (ChemBioChem/Wiley-VCH).

This paper addresses the issue of "Quorum Sensing", a phenomenon also present in *Pseudomonas aeruginosa* and other disease causing bacteria.

Quorum sensing is a system of communication bacteria uses to facilitate the coordination of gene expression of a number compounds and in *Pseudomonas aeruginosa,* a number of these compounds or virulence determinants are produced via a coordinated, cell- density dependent fashion achieved through a cell-to-cell signalling process.
The quorum sensing system of *P. aeruginosa* consists of an acylated homoserine signal molecule (autoinducer) and an autoinducer-dependent transcriptional activator protein (R protein).
P. aeruginosa produces a basal level of autoinducer whose concentration increases as its population grows (Pesci *et al.* 1999; Winzer and Williams, 2001; Van Delden and Iglewski, 1998; Camara *et al.* 2002; Erickson *et al.* 2002;). The autoinducer, which allows communication between *P. aeruginosa* population, binds to and thereby activates an R protein which subsequently induces or ceases to repress the expression of specific target genes.
To be successful , a pathogen must be able to sense and monitor the expression of genes necessary to establish itself in a new niche (Passador *et al.* 1993; Whiteley *et al.* 1999), quorum sensing provides this ability to *P. aeruginosa.*
The cell-to-cell signalling system of *P. aeruginosa* include the *las, rhl* and the PQS signalling systems.

- The *las* system consists of the autoinducer N-(3-oxo- dodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) synthesised by the autoinducer synthase gene *lasI* and the *lasR* gene that codes for a transcriptional activator protein. Activation of *lasR* by *lasI* leads to the induction of a *lasB* gene which encodes for the elastin-hydrolyzing protease LasB gene. The *las* system in addition to regulating *lasB* expression is also required for optimal production of other extracellular virulence factors such as LasA protease and exotoxin A.
- The *rhl* system consists of N-butyryl –L-homoserine lactone (C4-HSL) its autoinducer synthesised by the *rhlI* synthase gene and *rhlR* gene, a gene encoding a transcriptional activator protein, this system regulates the expression of the *rhlAB* operon that encodes a rhamnosyltransferase required for rhamnolipid production. The *rhl* system is also necessary for the optimal production of LasB elastase, LasA protease, pyocyanin, cyanide, and alkaline protease (Reimmann *et al.* 1997; Latifi *et al.* 1995; Brint and Ohman, 1995; and Pearson *et al.* 1997), the *P. aeruginosa* cell-to-cell signals (las and *rhI)* are both involved in the regulation of LasB virulence factor production , a third molecule was also found to be capable of its induction, this third molecule was called PQS.
- PQS (3,4-dihydroxy-2-heptylquinoline) whose precursor is HHQ (4-hydroxy-2-heptylquinoline) has been shown to modulate AHL-mediated quorum sensing in *P. aeruginosa* by providing a link between the *LasI* and *rhI* systems, PQS has been demonstrated to be a signalling molecule involved in cell-to-cell communication (Deziel *et al.* 2004).

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"Nasal Inoculation of the Commensal *Neisseria lactamica* Inhibits Carriage of *Neisseria meningitidis* by Young Adults: A Controlled Human Infection Study"

The authors (Deasy *et al.*, 2015) demonstrated that Non-pathogenic Neisseria could inhibit Meningococcal disease bacteria, their findings suggest that *N. lactamica* may one day help suppress meningococcal outbreaks as a bacterial medicine

These authors (Johnson *et al.*, and Deasy *et al.*, 2015) could demonstrate from their experiment that some important residues are needed by bacteria to start off an attack on a host and in addition how important avirulent non-pathogenic bacteria strains are in the area of drug discovery, I do not know how they intend to further carry on with their work but my opinion is that 'this is the direction' Scientific experiments of this century should be heading. Please fellow Scientists should read my propositions and see how we can help redirect the focus on the issues of drug discovery and antibiotic resistance.

Knowing what pathogens need to establish themselves (Osayande JO, 2015) or what they need to carry out an infection is an area of special interest in drug discovery.

It is time we start treating pathogenic organisms as humans (Osayande,JO, 2013), in this paper, I tried comparing *Pseudomonas aeruginosa*, to an individual based on my findings (see reference paper Osayande, JO, 2013).

Scientists today talk about antibiotic resistance. We would agree that apart from the issue of the abuse of antibiotics which is a human factor and a source of major concern, other important factors are being neglected. Factors like advocating the use of (for instance) hand gloves or hand sanitizers to minimize disease spread, personal hygiene, leaving pathogens where they first surface …. and so on are never written or spoken about the way they should be. It is not only during cases of disease outbreak hygienic advices should be given, it should be an everyday practice.

In Nigeria, during the Ebola virus disease outbreak in 2014, hand sanitizers were selling fast, Nigerians were able to contain and curtail the virus. A Visit to Nigeria today shows the contrary, hand sanitizers are no longer talked about (this information I gathered from the Nigerian local Newspapers). So sad, this is not the way to prevent disease spread. Hygiene should be an innate characteristic of the human, it will help Scientists a lot, because minimizing disease spread or pathogen transmission will probably (if not surely) minimize the emergence of new variants or outbreaks in places where they are not initially found. It takes little or no effort for diseases to break out but it takes the Scientist decades to find lasting solutions to combat disease.

The "boy meets girl" or "man meets woman" circumstance should give us an insight of what happens when bacteria or pathogens are trafficked from one place to the other. If bacteria were to be human, whatever transpires between a boy and a girl or rather a man and a woman should be exactly what to be expected between bacteria or other pathogenic organisms.

Scientific knowledge makes us understand that bacteria also carry out genetic material exchange. Furthermore we are told that through the processes of horizontal gene transfer bacteria can acquire genes or genomic islands (or distinguishably referred to as pathogenicity

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Islands, Kaper and Hacker, 1999) when they come in contact with other groups of bacteria or pathogens .

Supposing the genes acquired are antibiotic resistance genes, what are we likely to expect? A Stronger and more equipped pathogen in terms of resistance to antibiotics, this is also true for the offspring of a man or woman or a boy and girl after making out , an offspring will have in addition to other things, a mix up of genetic material . In the pathogenic world, these offsprings are termed variants, they are not new, the variant is just the same bacteria that has acquired something from another strain and this something is making it stronger in its defense ability.

A pathogen being trafficked from an "A" part of the world to a "B" part for example can come in contact with the pathogens in the B part of the world other to form a new pathogen, a variant form of the one coming from the A and B worlds respectively. Imagine that Scientists are already trying very hard to kill the strains from A and B worlds, if the A world strains are then successfully trafficked to B world, Scientists in addition to developing antibiotics for A and B strains, should also be preparing to develop antibiotics against the emerging variant "C" strains which are more difficult to eliminate because of the extra something that have been acquired from a new neighbor . If Scientists are able to find this extra "something" that has been acquired, then whatever gene or plasmid it is can be the focus or direction towards where a new drug is going to be synthesized or rather a modification of an existing drug based on the new gene that been acquired.

The emergence of variants or "offspring" can be reduced or prevented if human beings can be enlightened on minimizing pathogen transmission. Pathogens do not have legs, they need human beings as vehicles to transport them. Ebola virus does not have legs yet it was able to travel from Guinea where it first surfaced in 1976 (the year I was born) to Nigeria (in 2014) and other parts of the world.
There shouldn't be surprises when Scientists start to talk about variants of the Ebola virus, sooner or later.

It will suffice to say at this point that variants are not new species of pathogens but rather they are offspring coming from pathogens that have been trafficked from one place to the other, pathogens that have succeeded in coming in contact with new friends or neighbors and in addition to coming into contact where able to steal or acquire some genes to make them stronger in their niche.If pathogens remain where they first surfaced, issues of antibiotic resistance will not be as difficult as they are now.

This era should be an era whereby efforts are intensified towards minimizing pathogen spread, let pathogens remain where they are, let disease-causing organisms remain where they first surface.

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